Applicant: Dan E. Robertson et al. Attorney's Docket No.: 09010-010003 / DIVER 1180-2

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## **AMENDMENT**

Please amend the above-captioned application as follows:

## In The Claims:

Please cancel claims 24 to 39, and 56 to 60, 64, 66, 86 and 87 without prejudice.

Please amend the claims as follows:

- 1. (Currently amended) An isolated or recombinant nucleic acid comprising a sequence selected from the group consisting of SEQ ID NOS:23, 24, 25, 26, 27, 28, 29, 30, 31, 32 and variants thereof having at least about 50% sequence identity to SEQ ID NOS:23, 24, 25, 26, 27, 28, 29, 30, 31 or 32 SEQ ID NO:26 and SEQ ID NO:29, and encoding a polypeptide having an esterase activity.
- 2. (Currently amended) An isolated <u>or recombinant</u> nucleic acid of claim 1, comprising a sequence selected from the group consisting of <del>SEQ ID NOS:23, 24, 25, 26, 27, 28, 29, 30, 31, 32</del> <u>SEQ ID NO:26, SEQ ID NO:29</u>, sequences substantially identical thereto, and sequences complementary thereto.
- 3. (Currently amended) An isolated <u>or recombinant</u> nucleic acid that hybridizes to a nucleic acid of claim 1 under conditions of high stringency.
- 4. (Currently amended) An isolated <u>or recombinant</u> nucleic acid that hybridizes to a nucleic acid of claim 1 under conditions of moderate stringency.
- 5. (Currently amended) An isolated <u>or recombinant</u> nucleic acid that hybridizes to a nucleic acid of claim 1 under conditions of low stringency.
- 6. (Currently amended) An isolated <u>or recombinant</u> nucleic acid having at least 55% <u>sequence identity homology</u> to the nucleic acid of claim 1 as determined by analysis with a sequence comparison algorithm.
- 7. (Currently amended) An isolated <u>or recombinant</u> nucleic acid having at least 60% <u>sequence identity</u> homology to the nucleic acid of claim 1 as determined by analysis with a sequence comparison algorithm.



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- 8. (Currently amended) An isolated <u>or recombinant</u> nucleic acid having at least 65% <u>sequence identity homology</u> to the nucleic acid of claim 1 as determined by analysis with a sequence comparison algorithm.
- 9. (Currently amended) An isolated <u>or recombinant</u> nucleic acid having at least 70% <u>sequence identity homology</u> to the nucleic acid of claim 1 as determined by analysis with a sequence comparison algorithm.
- 10. (Currently amended) An isolated <u>or recombinant</u> nucleic acid having at least 75% <u>sequence identity homology</u> to the nucleic acid of claim 1 as determined by analysis with a sequence comparison algorithm.
- 11. (Currently amended) An isolated <u>or recombinant</u> nucleic acid having at least 80% <u>sequence identity homology</u> to the nucleic acid of claim 1 as determined by analysis with a sequence comparison algorithm.
- 12. (Currently amended) An isolated <u>or recombinant</u> nucleic acid having at least 85% <u>sequence identity homology</u> to the nucleic acid of claim 1 as determined by analysis with a sequence comparison algorithm.
- 13. (Currently amended) An isolated <u>or recombinant</u> nucleic acid having at least 90% <u>sequence identity</u> homology to the nucleic acid of claim 1 as determined by analysis with a sequence comparison algorithm.
- 14. (Currently amended) An isolated <u>or recombinant</u> nucleic acid having at least 95% <u>sequence identity</u> homology to the nucleic acid of claim 1 as determined by analysis with a sequence comparison algorithm.
- 15. (Currently amended) The isolated <u>or recombinant</u> nucleic acid of claim 1, 2, 6, 7, 8, 9, 10, 11 or 12, wherein the sequence comparison algorithm is FASTA version 3.0t78 with the default parameters.
- 16. (Currently amended) An isolated <u>or recombinant</u> nucleic acid comprising at least 10 consecutive bases of a sequence selected from the group consisting of <u>as set forth in SEQ ID NO:26 or SEQ ID NO:29 SEQ ID NOS:23, 24, 25, 26, 27, 28, 29, 30, 31, 32, at least 10 sequence selected from the group consisting of as set forth in <u>SEQ ID NO:26 or SEQ ID NO:29 SEQ ID NOS:23, 24, 25, 26, 27, 28, 29, 30, 31, 32, at least 10 sequence selected from the group consisting of as set forth in <u>SEQ ID NO:26 or SEQ ID NO:29 SEQ ID NOS:23, 24, 25, 26, 27, 28, 29, 30, 31, 32, at least 10 sequence selected from the group consisting of as set forth in <u>SEQ ID NO:26 or SEQ ID NO:29 SEQ ID N</u></u></u></u>



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consecutive bases of a sequence having at least 70% identity to SEQ ID NO:26 or SEQ ID NO:29 and encoding a polypeptide having an esterase activity, or sequences substantially identical thereto, and sequences complementary thereto.

- 17. (Currently amended) An isolated <u>or recombinant</u> nucleic acid having at least about 50% <u>sequence identity homology</u> to the nucleic acid of claim <u>16</u> <del>10</del> as determined by analysis with a sequence comparison algorithm or FASTA version 3.0t78 with the default parameters.
- 18. (Currently amended) An isolated <u>or recombinant</u> nucleic acid having at least about 55% <u>sequence identity homology</u> to the nucleic acid of claim <u>16</u> <del>10</del> as determined by analysis with a sequence comparison algorithm or FASTA version 3.0t78 with the default parameters.
- 19. (Currently amended) An isolated <u>or recombinant</u> nucleic acid having at least about 60% <u>sequence identity homology</u> to the nucleic acid of claim <u>16</u> 10 as determined by analysis with a sequence comparison algorithm or FASTA version 3.0t78 with the default parameters.
- 20. (Currently amended) An isolated <u>or recombinant</u> nucleic acid having at least about 65% <u>sequence identity homology</u> to the nucleic acid of claim <u>16</u> 10 as determined by analysis with a sequence comparison algorithm or FASTA version 3.0t78 with the default parameters.
- 21. (Currently amended) An isolated <u>or recombinant</u> nucleic acid having at least <u>about 70% sequence identity homology</u> to the nucleic acid of claim <u>16 10</u> as determined by analysis with a sequence comparison algorithm or FASTA version 3.0t78 with the default parameters.
- 22. (Currently amended) An isolated <u>or recombinant</u> nucleic acid encoding a polypeptide having a sequence selected from the group consisting of <del>SEQ ID NOS:33, 34, 35, 36, 37, 38, 39, 40, 41, 42</del> <u>SEQ ID NO:36 and SEQ ID NO:39</u>, and-sequences-substantially identical thereto.



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23. (Currently amended) An isolated <u>or recombinant</u> nucleic acid encoding a polypeptide comprising at least 10 consecutive amino acids of a polypeptide having a sequence selected from the group consisting of SEQ ID NOS:33, 34, 35, 36, 37, 38, 39, 40, 41, 42 SEQ ID NO:36 and SEQ ID NO:39, and sequences substantially identical thereto.

24 to 39. (Currently canceled)

- 40. (Currently amended) A method of producing a polypeptide having <u>an</u> esterase activity a sequence selected from the group consisting of SEQ ID NOS:33, 34, 35, 36, 37, 38, 39, 40, 41, 42, and sequences substantially identical thereto comprising introducing a nucleic acid <u>as set forth in claim 1</u> encoding the polypeptide into a host cell under conditions that allow expression of the <u>nucleic acid to produce a polypeptide</u> and recovering the polypeptide.
- 41. (Currently amended) A method of producing a polypeptide comprising at least 10 amino acids of a sequence as set forth in SEQ ID NO:36 or SEQ ID NO:39 selected from the group consisting of SEQ ID NOS: 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, and sequences or at least 10 amino acids of a sequence encoded by a nucleic acid as set forth in claim 1, substantially identical thereto comprising introducing a nucleic acid encoding the polypeptide, operably linked to a promoter, into a host cell under conditions that allow expression of the polypeptide and recovering the polypeptide.
- 42. (Currently amended) A method of generating a variant comprising: obtaining a nucleic acid comprising a sequence as set forth in SEQ ID NO:26 or SEQ ID NO:29, or a sequence as set forth in claim 1, or selected from the group consisting of SEQ ID NOS: 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, sequences substantially identical thereto, sequences complementary thereto, or fragments comprising at least 30 consecutive nucleotides thereof, and or fragments comprising at least 30 consecutive nucleotides of the sequences complementary to SEQ ID NO:26 or SEQ ID NO:29 SEQ ID NOS: 23, 24, 25, 26, 27, 28, 29, 30, 31 or 32; and

modifying one or more nucleotides in said sequence to another nucleotide, deleting one or more nucleotides in said sequence, or adding one or more nucleotides to said sequence.

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43. (Original) The method of claim 42, wherein the modifications are introduced by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis and any combination thereof.

- 44. (Original) The method of claim 42, wherein the modifications are introduced by error-prone PCR.
- 45. (Original) The method of claim 42, wherein the modifications are introduced by shuffling.
- 46. (Original) The method of claim 42, wherein the modifications are introduced by oligonucleotide-directed mutagenesis.
- 47. (Original) The method of claim 42, wherein the modifications are introduced by assembly PCR.
- 48. (Original) The method of claim 42, wherein the modifications are introduced by sexual PCR mutagenesis.
- 49. (Original) The method of claim 42, wherein the modifications are introduced by in vivo mutagenesis.
- 50. (Original) The method of claim 42, wherein the modifications are introduced by cassette mutagenesis.
- 51. (Original) The method of claim 42, wherein the modifications are introduced by recursive ensemble mutagenesis.
- 52. (Original) The method of claim 42, wherein the modifications are introduced by exponential ensemble mutagenesis.
- 53. (Original) The method of claim 42, wherein the modifications are introduced by site-specific mutagenesis.



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54. (Original) The method of claim 42, wherein the modifications are introduced by gene reassembly.

55. (Original) The method of claim 42, wherein the modifications are introduced by gene site saturated mutagenesis.

56 to 60. (Currently canceled)

61. (Currently amended) A method for comparing a first sequence to a reference sequence wherein said first sequence is a nucleic acid sequence as set forth in SEQ ID NO:26 or SEQ ID NO:29 or selected from the group consisting of SEQ ID NOS: 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, and sequences substantially identical having at least 50% sequence identity thereto, or a polypeptide sequence as set forth in SEQ ID NO:36 or SEQ ID NO:39 or selected from the group consisting of SEQ ID NOS: 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, and sequences substantially identical having at least 50% sequence identity thereto comprising:

reading the first sequence and the reference sequence through use of a computer program which compares sequences; and

determining differences between the first sequence and the reference sequence with the computer program.

- 62. (Original) The method of claim 61, wherein determining differences between the first sequence and the reference sequence comprises identifying polymorphisms.
- 63. (Currently amended) A method for identifying a feature in a sequence <u>as set</u> forth in SEQ ID NO:26 or SEQ ID NO:29 or wherein the sequence is selected from the group consisting of SEQ ID NOS: 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, sequences substantially identical having at least 50% sequence identity thereto, or a polypeptide sequence <u>as set forth in SEQ ID NO:36 or SEQ ID NO:39 or selected from the group consisting of SEQ ID NOS: 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, and sequences substantially identical having at least 50% sequence identity thereto comprising:</u>

reading the sequence through the use of a computer program which identifies features in sequences; and

identifying features in the sequences with the computer program.

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## 64. (Currently canceled)

65. (Currently amended) A method of catalyzing the hydrolysis of <u>an ester esters</u> comprising contacting a sample containing <u>an</u> esterase with a polypeptide <u>encoded by a sequence</u> as set forth in claim 1 selected from the group consisting of SEQ ID NOS: 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, and sequences having at least 50% homology and having esterase enzyme activity under conditions which facilitate the hydrolysis of <u>the ester</u> esters.

## 66. (Currently canceled)

- 67. (Currently amended) A nucleic acid probe comprising an oligonucleotide from about 10 to 50 nucleotides in length or at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150 or 200 nucleotides in length and having an area of at least 10 contiguous nucleotides having that is at least 50% sequence identity complementary to a nucleic acid as set forth in claim 1 or its complementary sequence target region of the nucleic acid sequence selected from the group consisting of SEQ ID NOS: 23, 24, 25, 26, 27, 28, 29, 30, 31 and 32, and which hybridizes to the nucleic acid target region under moderate or to highly stringent conditions to form a detectable target:probe duplex.
  - 68. (Original) The probe of claim 67, wherein the oligonucleotide is DNA.
- 69. (Currently amended) The probe of claim 67, which is having at least 55% sequence identity complementary to the nucleic acid target region.
- 70. (Currently amended) The probe of claim 67, which is having at least 60% sequence identity complementary to the nucleic acid target region.
- 71. (Currently amended) The probe of claim 67, which is having at least 65% sequence identity complementary to the nucleic acid target region.
- 72. (Currently amended) The probe of claim 67, which is having at least 70% sequence identity complementary to the nucleic acid target region.
- 73. (Currently amended) The probe of claim 67, which is having at least 75% sequence identity complementary to the nucleic acid target region.

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74. (Currently amended) The probe of claim 67, wherein the oligonucleotide comprises a sequence which is having at least 80% sequence identity complementary to the nucleic acid target region.

- 75. (Currently amended) The probe of claim 67, which is having at least 85% sequence identity complementary to the nucleic acid target region.
- 76. (Currently amended) The probe of claim 67, wherein the oligonucleotide comprises a sequence which is having at least 90% sequence identity complementary to the nucleic acid target region.
- 77. (Currently amended) The probe of claim 67, which is having at least 95% sequence identity complementary to the nucleic acid target region.
- 78. (Currently amended) The probe of claim 67, which is fully complementary to the nucleic acid target region.
- 79. (Original) The probe of claim 67, wherein the oligonucleotide is 15-50 bases in length.
- 80. (Original) The probe of claim 67, wherein the probe further comprises a detectable isotopic label.
- 81. (Original) The probe of claim 67, wherein the probe further comprises a detectable non-isotopic label selected from the group consisting of a fluorescent molecule, a chemiluminescent molecule, an enzyme, a cofactor, an enzyme substrate, and a hapten.82.
- 82. (Currently amended) A nucleic acid probe comprising an oligonucleotide from about 10 to 50 nucleotides in length or at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150 or 200 nucleotides in length and having an area of at least 15 contiguous nucleotides having that is at least 90% sequence identity complementary to a nucleic acid as set forth in claim 1 or its complementary sequence target region of the nucleic acid sequence selected from the group consisting of SEQ ID NOS: 23, 24, 25, 26, 27, 28, 29, 30, 31 and 32, and which hybridizes to the nucleic acid target region under moderate or to highly stringent conditions to form a detectable target:probe duplex.

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83. (Currently amended) A nucleic acid probe comprising an oligonucleotide from about 10 to 50 nucleotides in length or at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150 or 200 nucleotides in length and having an area of at least 15 contiguous nucleotides having that is at least 95% sequence identity complementary to a nucleic acid as set forth in claim 1 or its complementary sequence target region of the nucleic acid sequence selected from the group consisting of SEQ ID NOS: 23, 24, 25, 26, 27, 28, 29, 30, 31 and 32, and which hybridizes to the nucleic acid target region under moderate or to highly stringent conditions to form a detectable target:probe duplex.

84. (Currently amended) A nucleic acid probe comprising an oligonucleotide from about 10 to 50 nucleotides in length or at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150 or 200 nucleotides in length and having an area of at least 15 contiguous nucleotides having that is at least 97% sequence identity complementary to a nucleic acid as set forth in claim 1 or its complementary sequence target region of the nucleic acid sequence selected from the group consisting of SEQ ID NOS: 23, 24, 25, 26, 27, 28, 29, 30, 31 and 32, and which hybridizes to the nucleic acid target region under moderate or to highly stringent conditions to form a detectable target:probe duplex.

85. (Currently amended) A polynucleotide probe for isolation or identification of esterase genes having a sequence which is the same as or fully complementary to at least a portion of <u>SEQ ID NO:26 or SEQ ID NO:29</u> <u>SEQ ID NOS: 23, 24, 25, 26, 27, 28, 29, 30, 31 or 32</u>.

86 and 87. (Currently canceled)

- 88. (Original) A method for modifying small molecules, comprising mixing a polypeptide encoded by a polynucleotide of claim 1 or fragments thereof with a small molecule to produce a modified small molecule.
- 89. (Original) The method of claim 88 wherein a library of modified small molecules is tested to determine if a modified small molecule is present within the library which exhibits a desired activity.



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90. (Original) The method of claim 89 wherein a specific biocatalytic reaction which produces the modified small molecule of desired activity is identified by systematically eliminating each of the biocatalytic reactions used to produce a portion of the library, and then testing the small molecules produced in the portion of the library for the presence or absence of the modified small molecule with the desired activity.

- 91. (Original) The method of claim 90 wherein the specific biocatalytic reactions which produce the modified small molecule of desired activity is optionally repeated.
- 92. (Original) The method of Claim 90 or 91 wherein (a) the biocatalytic reactions are conducted with a group of biocatalysts that react with distinct structural moieties found within the structure of a small molecule, (b) each biocatalyst is specific for one structural moiety or a group of related structural moieties; and (c) each biocatalyst reacts with many different small molecules which contain the distinct structural moiety.

Please add the following new claims:

- 93. (NEW) An isolated or recombinant nucleic acid having at least about 75% sequence identity to the nucleic acid of claim 16 as determined by analysis with a sequence comparison algorithm or FASTA version 3.0t78 with the default parameters.
- 94. (NEW) An isolated or recombinant nucleic acid having at least about 80% sequence identity to the nucleic acid of claim 16 as determined by analysis with a sequence comparison algorithm or FASTA version 3.0t78 with the default parameters.
- 95. (NEW) An isolated or recombinant nucleic acid having at least about 85% sequence identity to the nucleic acid of claim 16 as determined by analysis with a sequence comparison algorithm or FASTA version 3.0t78 with the default parameters.
- 96. (NEW) An isolated or recombinant nucleic acid having at least about 90% sequence identity to the nucleic acid of claim 16 as determined by analysis with a sequence comparison algorithm or FASTA version 3.0t78 with the default parameters.

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97. (NEW) A isolated or recombinant nucleic acid at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150 or 200 consecutive residues of a nucleic acid as set forth in claim 1.

98. (NEW) A vector comprising a nucleic acid as set forth in claim 1 or claim 97.

99. (NEW) The vector of claim 98 comprising a wherein the vector comprises a viral particle, a baculovirus, a phase, a plasmid, a cosmid, a fosmid, a bacterial artificial chromosome, a viral DNA or a P1-based artificial chromosome.

100. (NEW) A host cell comprising a nucleic acid as set forth in claim 1 or claim 97.

101. (NEW) The host cell of claim 100 comprising a eukaryotic cell or a prokaryotic cell.

102. (NEW) The host cell of claim 101 comprising a plant cell, a mammalian cell, a fungal cell, a bacterial cell, a yeast cell or an insect cell.

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